



100 days in the life of a tsetse fly

The tsetse fly tells her story of how she became an involuntary vector transmitting trypanosomes. These parasites cause Nagana disease in cattle throughout sub-Saharan Africa and sleeping sickness in humans in some thirty African countries.

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Authors:

Georgette CHARBONNIER, Michel LAUNOIS,

with special contributions from:

Reza BENTALEB, Jérémy BOUYER, Emmanuel CAMUS, Jean-François DEBERNARD, Stéphane DE LA ROCQUE, Gérard DUVALLET, Jean-Louis JACQUEMIN, Hamadi KAREMBE, Renaud LANCELOT, Martin MITCHELL, Anne HOLM RANNALEET, Michel TRANIER,

and the support of:

Denis BASTIANELLI, Pierre-Marie BORNE, Marie-Françoise BARRET-MARGER, Florence CAVILLAC, Stéphane COLSENET, Eduardo Evaristo DE MIRANDA, Jean-Pierre DUBOIS, Roberto DE MIRANDA, Bernard FAYE, Abdoulaye GOURO, Olivier LAS VERGNAS, Charlotte LAVESON, Vincent PORPHYRE, Marc PRIKAZSKY, Anita THIOUX, Jeanne SUHAMY, Charles WAISWA.

Original drawings:

Thanh Minh LUONG.

Photographs:

Reza BENTALEB, Jérémy BOUYER, Olivier ESNAULT, Anne HOLM RANNALEET.

Graphics and Layout:

Géraldine LAVEISSIERE.

Translation into English:

Grace DELOBEL.

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Published with the support of Ceva Santé Animale and IKARE, this scientific animal tale about the tsetse fly is not meant for sale. It is meant to be distributed among diverse audiences with the aim of contributing to the dissemination of scientific knowledge and to support educational projects reaching the greatest number of people possible. Primarily aimed at those whose lives are directly or indirectly touched by sleeping sickness and hence the tsetse fly.

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Introduction

This story, the fruit of the imagination of scientists and practitioners, narrates the intimate life of a blood-sucking African insect: the tsetse fly.

Weaving together knowledge validated by entomologists, parasitologists, veterinarians, and doctors, this animal empathy experiment invites you to share, for the duration of the tale, the ordinary life of a female tsetse fly from birth to death.

To enter into the story, imagine that you are in a humid, tropical area somewhere in Sub-Saharan Africa, and that you are an egg in the uterus of a tsetse fly -- your mother -- and let yourself live...

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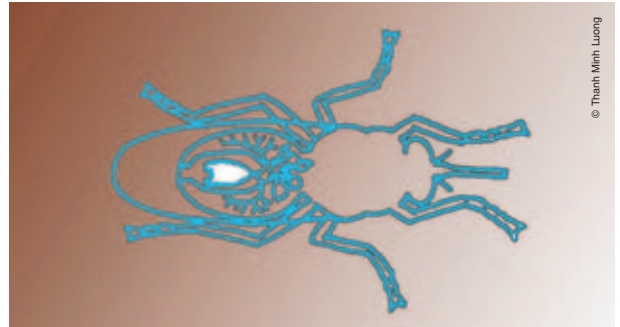
A disease shared between animals and humans

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■ The encapsulated egg

My father and my mother are male and female insects that each produce distinct sex cells, or gametes. The egg from which I will emerge is born of the fusion of a cell from my mother, the mature follicle, and a cell from my father, the spermatozoon. The meeting takes place just outside the opening of the spermathecae duct. Spermathecae are organs located in my mother's abdomen where male semen are stored and conserved.

In contrast to other flies, which tend to be more wasteful but more fecund, capable of laying several hundreds of eggs at a time, the egg is not expelled from the uterus. Tsetse flies practice viviparity, meaning embryos and larvae develop within our mother's bodies.



My position in the uterus of my mother.

I spend my first days of existence in the form of an egg lodged in the uterine cavity of my mother, which does not interfere in the slightest with her flying.

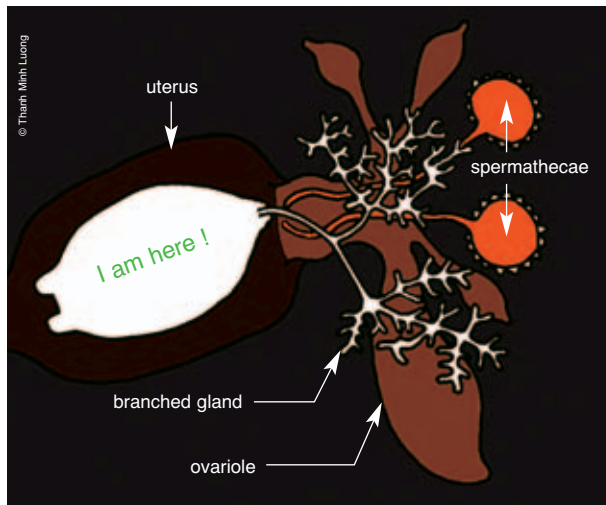
In this position, I feel her shifts in position, accelerations and decelerations, as well as temperature changes between day and night. My cells divide rapidly and the rough draft of my future organs settles into place following a precise anatomical plan. At the end of three days of forced incubation, when the development of an elementary nervous system is achieved, I have become a very young larva, known as a first instar and easily hatch myself from the envelope of the egg.

It is in this form, which resembles a whitish maggot, that I am brought into the world when the time is due.

From the point of view of the species, this mode of birth ensures the best chances of survival and compensates for the low fertility of our mothers. However, the reverse may be true, meaning that their low fertility obliges them to lavish great care on each of their young.

■ A three-stage larva

Still sheltered inside my mother's abdomen, I instinctively find the teat-like end of the branched uterine gland which juts out from the ceiling towards the front of the uterus, precisely where I position my head. The milky secretions that seep out are as nourishing as the residual vitellus stored in my digestive tract. I ingest along with these secretions at least two types of intestinal bacteria, *Wigglesworthia glossinidae* and *Sodalis glossinidius*. These will provide me with group B vitamins and pantothenic acid, crucial for my own future fertility.



My position in relation to the reproductive system.

After a day and a half of life, I clearly feel changes taking place in my body. Two large respiratory trunks, the trachea, are differentiating themselves laterally in my abdomen. They rejoin the respiratory spiracles placed near the rear end of my body. The tracheoles located on the tips of my trachea branches can now allow the oxygenation of new organs. Although I am still very weak, I can move enough to achieve my first larval moult, meaning that I shed my first larval skin.

Now a second instar, I pursue my development within my mother's abdomen, sheltered from the terrible maggot predators lurking in the outside world. At this stage in my life, my exterior shape evolves. Two swellings appear around the air intakes called polypneustic lobes, placed at the rear end of my abdomen. I still keep my head near the teat, maintaining a position in line with that of my mother. She adjusts her abdominal muscles to adapt the size of her uterus to my growth. After two or three days, I sense the time has come to moult again and shed my second larval skin.



During transformation:
From the second (left) to the third instar (right).

Now a third instar, I busy myself with stocking fat reserves for the future. My posterior swellings harden and assume a brown colour. In another three days, I will be ready to be born. My mother feels this and reacts with spasmodic uterine contractions. The delivery is

■ A ten second birth

The ten days which have passed between the formation of my egg and the end of my larval life have been very busy. The crucial moment has now arrived. My mother takes a horizontal position, probably on the ground because the air that is reaching me through her vagina has an earthy odour. She is agitated by the uterine contractions, which I try to accompany as best I can by wriggling, up to the moment when I feel myself being expelled from the uterus. I suddenly come into contact with warm, humid soil.



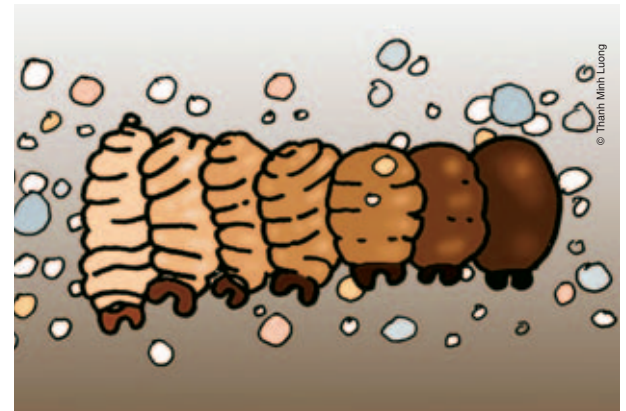
The delicate stage of my birth.

I am in shock. Out of surprise, I emit an odour through my anus that could serve as an indicator of a birth site to other pregnant females, meaning ones ready to give birth. My biological mother undoubtedly notices my moment of hesitation. By rapidly tapping me on the back with her hind legs, she encourages me to react. I comply. I reflexively burrow into the sandy-clay soil with my posterior shelled ridges, wriggling vigorously, filled now with a sense of urgency. In a few minutes, my 8 millimetre long, whitish body disappears beneath the surface. I wriggle another few centimetres down into the earth. My passage from uterus to humus is successful.

I gradually relax and wriggle around to make myself comfortable in my new home. A few seconds later, I feel a vibration produced by a 2000 kHz sound emitted by my mother which resonates for several minutes. Silence falls. And then I hear her take off. I will never know whether it is a cry of farewell, or a way to signal to other pregnant females that this third instar larva deposit spot is perfect, or simply a sign of jubilation for having successfully completed her pregnancy. Might it have been the first?

■ The pupa in place

Once the dust has settled, I immobilize myself safely out of sight, my head pointing towards the surface of the ground. My exterior cuticle is rapidly hardening, becoming the same black colour as my posterior swellings. At the same time, my epidermis is becoming impermeable. I will soon feel sufficiently safe within this new, barrel shaped shell, the puparium, to undertake a radical reorganization of my organs, like other flies do. Nothing in my current state could provide a clue as to what I am to become. I am obeying a metamorphosis that has been programmed entirely by my genes.



A rapid evolution into the pupa state.

This particular period of my life, called the pupal or sometimes nymphal stage, can last from 20 to 80 days depending on a fly's species and sex, as well as the season and ambient conditions in this part of Africa. In my case, pupation takes 40 days, during which all of the organs in my body undergo profound and troubling changes. It is as if each cell of every one of my organs is unleashing itself to assume another position in accordance with a new internal design plan.



Like a capsule in the ground.

At times I am not even conscious of being alive. At this stage of the metamorphosis, an overly curious entomologist who is fond of vivisection would find beneath his knife only a whitish culture of cells with no visible trace of organization into organs. Everything that made me a larva has been overthrown.

During this period of immobility and metamorphosis, the fat reserves stored during my time as a larva are my sole source of nourishment. Luckily, they are plentiful, because my transformation into an adult is taking place with no other intake of food. This particular feature, which is unique in the animal world, is characteristic of holometabolous insects, of which I am one.

When once again I have the impression that I own my whole body, I feel as if I am reborn. With the loss of the memory of what I once was, I have become a perfectly formed tsetse fly, obsessed with the pressing need to escape as quickly as possible from the rigid puparium. I feel horribly confined.



Isolated and confined, I transform myself.

■ The very young fly

To free myself, I discover three things:

- on my head, there is a kind of projection, the ptilinum, which could be used as a ram;
- there seems to be a pre-cut circular cap on the upper part of my puparium;
- I can make my interior liquid, the hemolymph, flow up to swell my head capsule, coordinate all of my muscles, and consequently, increase the pressure on the pre-cut cap which seems to be made to open easily at this stage of my life. This is a trait that makes us, tsetse flies, part of the dipteran cyclorrhapha suborder.

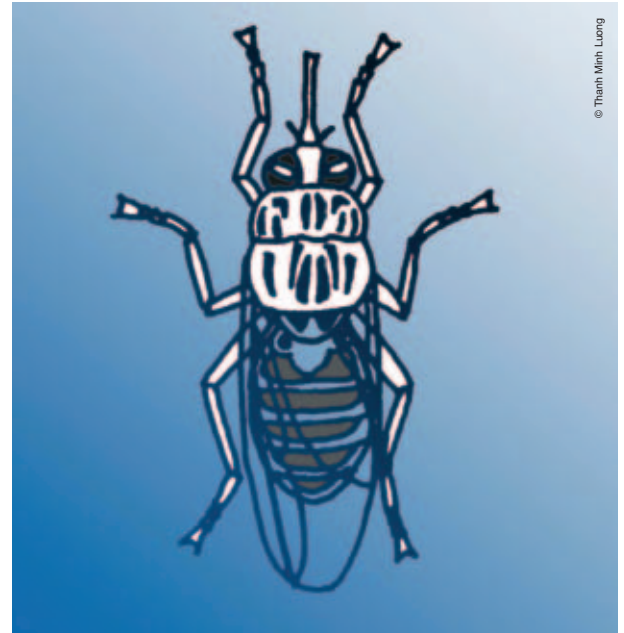
Being very determined, it only takes me a few seconds to extract myself from this vestigial envelope and wriggle to the surface, leaving behind a kind of empty tunnel. Detritivorous insects and bacteria specialized in processing chitin will probably make good use of it.

I am now a tender young fly known as a teneral, due to my soft body. I will continue to bear this name until I take my first blood meal.



The first phase of liberty.

While wandering about in search of a perch, I inflate my abdomen and fill my new tracheal network with air. My wings then unfold as their veins start to swell. My brand new proboscis stretches out horizontally.



Becoming a perfect insect.

A few steps away, a fellow fly is being attacked by a gang of aggressive ants. This fact leaves me completely indifferent. I like to be alone because I am one of those flies that enjoy solitary life. The reserves of fat that I did not use during my existence as a larva or a nymph increase my chances of survival as an adult and give me some time to discover appropriate food. I rest for an hour hidden in the underbrush, half in the sun, half in the shade. I must discretely complete my preparations for life above ground. My muscles are gradually strengthening themselves. Their anchors on the endoskeleton's apodemes are becoming firm.

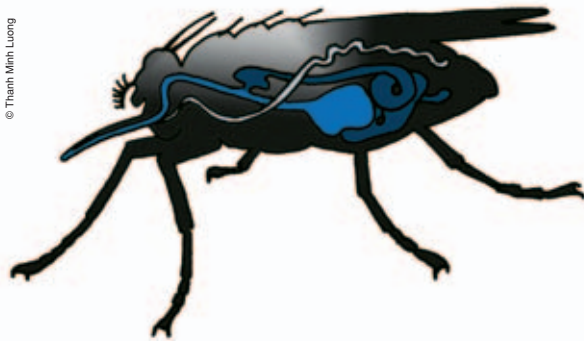
■ A fly different from other flies

One centimetre long from the tips of my antennas to the ends of my two wings folded over my back, there is nothing remarkable about my sleek silhouette. My body is robust, but nothing more. A little heavier than a male of my species, I must weigh about 12 milligrams when my crop is almost empty. I am brown, without any bright colours.

But I am different from the 85,000 other species of flies in a number of ways, and I can claim a distinctive name: *Glossina palpalis gambiensis*, which a scientist gave me back in 1949.

According to current taxonomy, we *Glossina palpalis gambiensis* are one of the 9 species in the palpalis group, which in turn belongs to the *Glossina* genus. All 31 species of this genus reside in Africa and are from the same family, the *Glossinidae*. It is an exclusive feature of the continent.

We all also make a sound that was recognized by the people of the Matebele and Zulu ethnic groups centuries ago; they nicknamed us “tsetse” due to the noise we produce while flying.



My digestive system, from the proboscis to the cloaca, with my long salivary glands.



The normal posture of the fly with a bad reputation.

My reputation among humans has never been very good; even pastoralists in ancient times knew me as a fly “deadly for animals and the young”. They were referring to Nagana disease in livestock and sleeping sickness in themselves. Yet my responsibility is neither as direct nor as important as it would seem, and the same is true for other tsetse flies. In fact, many of us live out our entire lives without ever carrying pathogenic parasites. Of course, I admit that for people the difference between a deadly fly and a fly that simply enjoys some blood may not be immediately evident. And even if I resist those horrible trypanosomes better than vertebrates, I do not choose to be infected by protozoan parasites any more than they do. I, too, fall ill.

For the moment, I am healthy and have no other immediate intention than to learn how to fly. Hidden under a leaf, in the shade, the air warm and humid, I am resting after having tried several times when my body was still too soft and limp. I am a day-time fly, and after considerable efforts, I master all of the manoeuvres between taking off and landing, including all swift changes in flight, which requires very good muscle and nerve coordination. My average speed is now 10 meters per second, or 36 km an hour. At the dawn of my third day of life, I already feel like a full adult and involuntarily emit my first sexual pheromones.

■ An assessment of my senses before the encounter

While waiting for a response to my hormonal secretions, I take an inventory of my six senses.

I have two large compound eyes placed at the front of my head. My eyes are formed by ommatidia, which allow me to detect the colours, movements, contrasts, forms and dimensions of all objects passing through my visual field, which I know is large. This is an advantage for a hunter, whose spirit I feel rising within me. I can detect the movement of frightened antelope at a distance of over 140 meters. At a distance of 20 meters, I can clearly distinguish a fifth instar larva of a multi-coloured cricket chewing on leaves as if it was all alone in the world. I easily perceive blue, red and green colours. I am attracted in particular by the wavelength of blue reflected light. In contrast, I avoid that of green, yellow and red. I also like the high reflectiveness of white and black in the ultraviolet field as well as contrasting tones. Humans know my preferences. They build traps with my three favourite colours (blue, white and black) to lure me in and capture me.

I also have three small, simple eyes, the ocelli, which are located at the base of my two short antennae and in the middle of my forehead respectively. Very sensitive to light, connected by nerve and perhaps endocrine paths to my internal circadian clock, they manage my overall health.

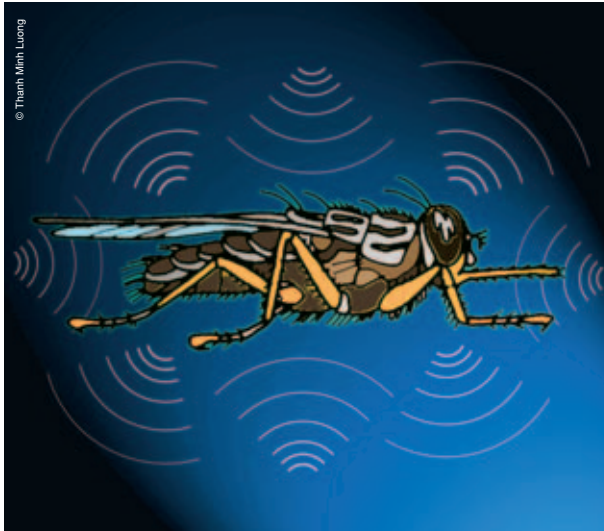
My sense of hearing is greater than one typically imagines. At this moment, for example, I am hearing the hum of a large hornet, the cries of some birds, and the cracking of a branch. More sensitive to deep sounds than squeaky ones, I can decode in priority the calls of the opposite sex, a gift from birth, I assume, that probably is reinforced by my hormonal state.

I detect smells thanks to numerous sensors known as chemoreceptors that are situated around my two antennae, my mouthparts, and my genitals. Each chemoreceptor is specialized in a specific range of odour molecules: ketone, aldehydes, phenols, carbon dioxide and even the pheromones of adult males and newborn larvae. My brain manages the odour map of my environment, at least for the fragrances which interest me. I am capable of tracing the gradient of an odour back to its source. Attracted by the smell of urine, excrement, sweat, fur, and the breath of mammals, my future prey, I instinctively sense that I am more of a hunter than a gatherer.



Equipped with smell, sight, touch, taste, and hearing-to-live as long as possible.

My taste chemoreceptors, which detect amino acids and uric acid, are located on my proboscis and on the tips of my six feet. Thus equipped, I have the sensory capacity to chemically analyse the lymph and blood which will be my future food. All that now remains is to find an occasion to put all of this to good use. Suddenly, the idea of a blood meal becomes part of my tsetse fly conception of the world.



A segmented body well equipped with sensors.

Hypersensitive mechanoreceptive bristles cover my body and supply me with touch information. I use them to detect the direction and strength of the wind, and the air flow on my forewings and around the remains of my hind wings, which have been transformed into kinds of gyroscopes. Among the diptera, the only visible pair of wings is the membranous forewings, which differentiate us from wasps and bees, hymenopterous insects that have four membranous wings. This fundamental difference does nothing to stop us from flying just as well or just as fast as they do, the only difference being hovering where we are slightly less at ease.

Mechanosensory pressure sensors implanted on all of my entire external skeleton warn me of the approach of undesirable predators such as chameleons and lizards, and allow me to hear certain very low frequency sounds.

Proprioceptors wisely placed on my intersegment membranes and a triple brain, the proto, deuto, and tritocerebrum, which are welded together, provide me with 3-D information on the relative position of my six jointed feet.

Scientists intrigued by the way I become agitated when the weather is stormy suspect that I also have a sixth sense that makes me able to exploit the polarization of light and to react to electromagnetic fields and variations in atmospheric pressure.

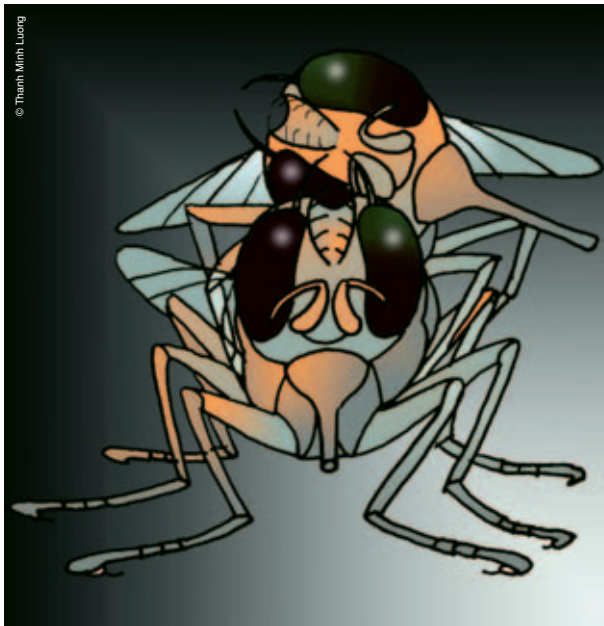


At rest, in the shade of a branch.

■ Mating before eating

Just as I am about to undertake a short, low altitude reconnaissance flight of 200 to 300 meters, I am approached by a young male who seems to appreciate the quality of my sex pheromones.

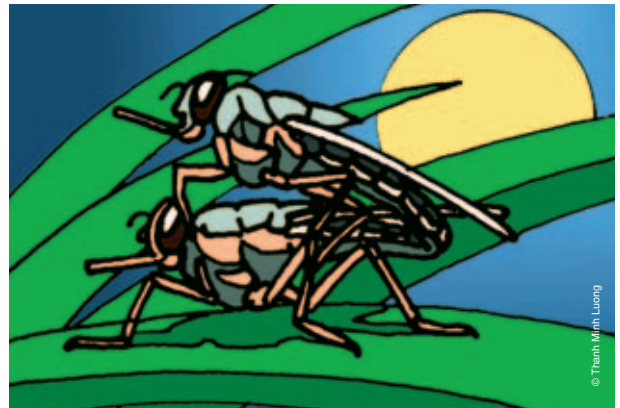
The male fly quickly places himself on top of my abdomen with a single flick of his wings. I reflexively spread my own wings to allow him to position himself better, which he does immediately, anchoring himself firmly against me with his hind feet. He fixes the tip of his abdomen to mine with two hinged claws made for this purpose, the claspers, which probably will leave scars on my abdomen. Even though it is quite painful, I remain still, as if paralysed.



A single mating with complex sensations.

Once calm and well wedged against me, this fellow fly unfolds his hypopygium, a male genital apparatus with telescopic qualities, and shifts it 180° down and forward to reach the underside of my abdomen. Securely positioned and confident, he then takes the time to fabricate spermatophores, which are kinds of sacks of live spermatozoa, before injecting them into the depths of my uterus, gently but with determination. All of this lasts at least an hour, which is relatively long for flies, many species mate only in passing, sometimes even in full flight.

His sperm-donor mission accomplished, he flies off, undoubtedly to couple with other females of my species. Mating with females of other species would in any event end in failure due to incompatible external genital organs. Natural selection keeps watch.



Discreetly fulfilling the duty to reproduce.

Several hours after this encounter, once the spermatozoa have been transferred successfully into my spermathecae, I eject the spermatophore, now emptied of their contents. The spermatozoa can remain alive in my sperm bank for at least 200 days, although my maximum lifespan is only 100 days on average. Nature is thus taking careful precautions. I will only use a miniscule amount during each ovulation to fertilize the oocyte produced.

Even if I occasionally accept being mounted by other males, these couplings will be infertile because the first ovulation on the 10th day of my winged life mechanically blocks their spermatozoa from reaching my spermathecae.

Female tsetse flies' ritual of a single mating, and the unrenewable stock of spermatozoa of our male fellows, could be fatal for our species. Scientists have for example proposed to control our population by inundating us with an army of males sterilized with radiation, thereby rendering our couplings infertile. As today, this method of controlling our survival has only been successful in geographically limited and restricted populations of tsetse flies, such as those living on the island of Zanzibar.

■ The vital blood meal

Now that my vital role to reproduce has begun, it is physiologically essential that I succeed in serving myself a vampire meal. It is our custom. The pursuit of our entire biological cycle depends on it: steady ovulation, successful pregnancies, completion of young imago growth and storing fat for energy consuming flight activities, without mentioning the putting aside of reserves for difficult times.

I therefore begin a quest for a host to satisfy my need for blood. After putting my entire sensory system into action, the smell of a humped zebu cow signals a possibility. For this first meal, I am not very picky. It will do.

On this beautiful sunny morning, I can distinguish the zebu's silhouette clearly. I identify the odours that the animal gives off, and I automatically calculate its travelling speed in order to choose the best interception trajectory. After a few short flights, I reach the target. Landing on the base of its front leg, I instinctively assume a position out of reach of the animal's tail.

The cow apparently does not notice any difference between my landing and that of the equally light landings of ordinary flies that bother him. Humans know our favourite attack areas, the lower limbs of ruminants and horses. They sometimes treat them with insecticides by having the animals walk through footbaths or by spraying them. Luckily for us, such health care practices remain rare in the bush.

Immediately, I take a horizontal position with my head down and spread my maxillary palps which are one of my mouth parts. I then vertically lower my proboscis, equipped with a rasp on its end, and cautiously thrust it into the skin up to its bulb to dilacerate the tissues and suck in the flow of blood. Probably due to a lack of experience, this first attempt fails. I extract my proboscis and probe around until I find the right spot. My bites appear to be practically painless because they do not disturb my host. She continues to graze, moving slowly. I am now ready to satisfy both my thirst and hunger in one go.



A healthy cow as the first target.

Moving the sharp, toothed end of my proboscis like a saw, I create a small hematoma under my host's skin while injecting anticoagulant saliva. I take about twenty seconds to suck in at least 50 milligrams of blood that I store in the crop of my abdomen. Only a small quantity passes from there directly into my digestive tract. The rest will be digested following a more complex process. Once satiated, I withdraw my proboscis, which automatically resumes a horizontal position. I now am twice as heavy as I was before eating.

To eat, I had to expose myself to unbearable heat that will be deadly if I do not quickly seek shelter. Therefore, I speed back to the protected area of the nearby forest to digest in peace, sheltered from the sun -- and from the eyes and teeth of my natural enemies.

■ My preferred habitat

Unable to tolerate low humidity or high temperatures, I live in a special plant environment in which I can find the microclimate that suits me best.

Deep in the forests, I hunt in mangrove biotopes and reproduce under the palm groves located behind the mangroves.

In the savannahs, my cousins live in woodland galleries growing beside waterways.

In other parts, they frequent the vegetation growing in depressions between dunes flooded by flushed water tables.

The common element shared by these habitats is the presence of fresh or brackish water needed for the growth of shade trees that effectively filter the hot African sun. As other live beings share the same needs, we often encounter our future prey in these areas.

■ Slow and thorough digestion

Those who imagine that blood is easy to digest have never known the life of a tsetse fly. My first step is to close my food canal to avoid dehydration. Although it is located in the forefront of my abdomen, the crop actually is a kind of pouch, or diverticulum of my oesophagus.



Source : Jérôme Bouyer

An abdomen full of blood from others.

Five minutes after the end of my meal, I feel contractions that push the blood towards the proventriculus (the part of my stomach) located at the level of my thorax. This valve pushes the blood into the midgut (the middle part of my intestines) where it is enclosed in a kind of protective sleeve, the peritrophic membrane, which can be permeated by digestive enzymes. This long sleeve is made continuously at a rate of 1 millimetre per hour from the first hour of adult life. My crop empties itself in about thirty minutes, but it takes approximately 80 hours for these blood bags to reach the hindgut. During the following three hours of digestion in the intestines, proteolytic enzymes transform the red blood into a black increasingly pasty material through gradual dehydration.

This first meal provides me with the amino acids required to consolidate my thoracic muscles and stimulate my ovaries. One amino acid, proline, is also my main flight fuel. Immediately after this snack, I excrete a clear drop from my anus, to eliminate the excess water in my liquid meal. Tiny amounts of sugar and fat energy molecules are stored in my fatty tissue.

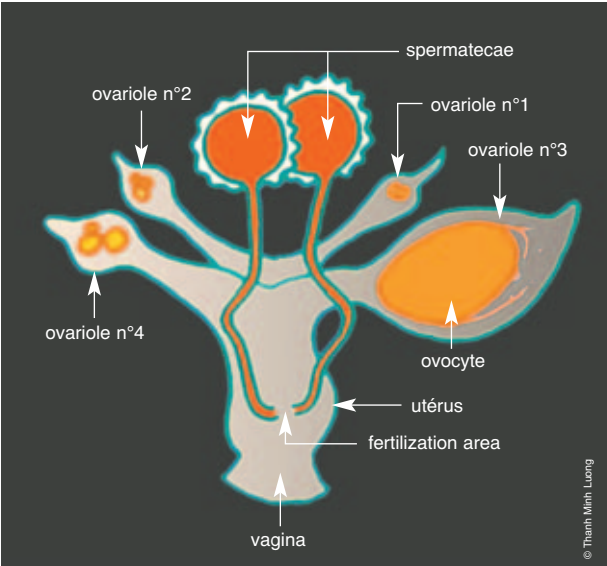
■ Four stroke ovaries

This blood meal triggers my first ovulation. I feel ready to take care of my offspring thanks to the highly original way that my ovaries function. My two ovaries have each one internal and one external ovariole. These four ovarioles take turns functioning every 10 days according to a precise order. For this first time, it is the internal ovariole in my right hand ovary that is activated and produces a follicle.

Although it began well, and environmental conditions were good, the development of this first egg is suddenly interrupted. The oocyte stops growing, the vitelline reserves are reabsorbed, and all that remains inside my ovariole are carotenoid waste that forms a resorption body. Could it be possible that the *Wigglesworthia glossinidae* bacteria, which were transmitted to me by my mother and stored in the special epithelial cells of my uterus, were not yet adequately organized to produce sufficient amounts of the molecules critical for the expression of my fertility? This question remains a biochemical mystery.

Without wasting time, the internal ovariole of my left ovary takes over. This time, the oocyte is a success. The ripe follicle is ready to be fertilized at the exact moment that it passes in front of the spermathecae. With a reflex contraction, the in utero egg laying is successful just 24 hours after the first aborted effort. The ovulation leaves at the base of the ovariole a whitish sleeve formed by the food cells of the oocyte, the follicular cells. This provides evidence of an egg's passage used by scientists to determine the age of a reproducing fly.

A few hours later, the external ovariole of the right ovary kicks into operation. Next, the external ovariole of the left ovary will take over, and finally the internal ovariole of the right ovary, and the cycle will continue in unchanging order. Each time, however, only one larva will reach its final stage of development.



Two spermathecae, four ovarioles, one uterus:
a functioning reproduction.

The young larva wriggling inside my uterus takes ten days to fully develop and become cumbersome. I therefore give birth to set the pulpa free, like my mother did for me, and emit the special 2000 kHz song of birth. This sound alerts other pregnant females that this location is ideal to perpetuate the species. It is our way of communicating.

■ A different meal

In our family, each tsetse fly species has specific food preferences. Some are very picky. They have very marked preferences and will only feed on the blood of swine such as domestic pigs, warthogs, and bushpigs, or on the blood of cattle.

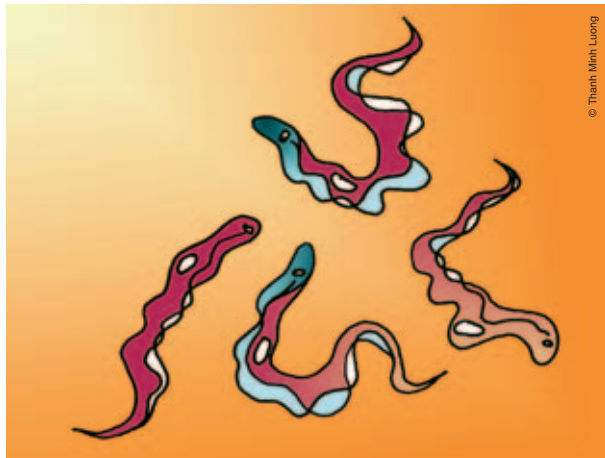
Others are more opportunistic and are happy to feed on the blood of domestic animals such as cattle, horses, camels, donkeys, that of wild animals such as elephants, rhinoceros, hippopotamus, aardvark, porcupines, crocodiles, snakes, and ostriches, and that of people. I belong to this category.



A weakness for placid cattle.

When I have the choice, I prefer placid hosts like cattle. I do not trust goats, which devote far too much energy in trying to stop us from having a decent meal, some going as far as trying to crush us with their mouths or feet. These muscular defensive movements teach us many lessons.

In addition to my topographic memory, which is very handy for finding the same roost several nights in a row, and for returning to the same hunting grounds frequented by my prey, I have a trophic memory. This gives older flies an ability to eat well which is superior to that of a young fly fresh out of the puparium. We learn from experience to increase our chances of survival. We even develop habits when these can make our lives easier and ensure a blood meal every 3 or 4 days.



Parasites in the blood taken from my cattle prey.

A few days have passed since my last meal and once again I am filled with a thirst for blood. My pregnancy does not interfere with my quest for food because filling my crop is not in competition with my pregnant uterus.

A cocktail of irresistible odours emanating from a cow saturates my sensory receptors, although the cow actually is not very physically attractive, thin with an indolent air and dull fur, normally a sign of some illness. I do not hesitate to draw blood. This second blood meal normally runs no risk of infection because during the first digestion, our intestinal enzymes produce lectins that render us resistant to trypanosomes, the parasites that can infect us.

However, in contrast with the last time I digested a meal, I rapidly start to feel uncomfortable, a discomfort that gradually will affect my activities, part of my behaviour, and my physiology. Too late, I realize that my prey was infected with acute trypanosomiasis, that I involuntarily ingested hundreds of trypanosomes in the blood, and that I was, in a rare twist of fate, still susceptible to trypanosomes.

The trypanosomes were not destroyed by my intestinal lectins while passing through the upper part of my midgut as they normally should have been. Could the reason be that there were too many of them or that the strain involved was particularly virulent? I don't know. Whatever the reason, I find myself in the situation of a young tsetse fly after a contaminated first blood meal, where the chitinases synthesised by symbiotic intestinal bacteria passed down to me by my mother while I was still in her uterus inhibits intestinal lectins from destroying the trypanosomes.

The parasites continue their invasion into the most intimate depths of my body. The protozoa traverse my intestinal tunic, migrate towards my hypopharynx and colonize my salivary glands. Without actually suffering, I feel a slight discomfort at the level of my abdomen, above my digestive tract, there where they have coiled around each other. My saliva, which had been transparent, becomes milky, its acidity diminished, its chemical composition modified. Some sugars disappear. A new phospholipid, probably produced by the parasites, appears. I am an involuntary vector and powerless host of undesirable guests that are ensuring their survival at my expense.

Despite this microbial invasion, with all of its cellular and enzymatic battles, I pursue my reproductive mission. However, each new egg is produced under increasingly unfavourable energy conditions. My time on earth is running out. Yet my infectious state seems to have stabilized, as if the 200,000 trypanosomes inside my body do not wish to see me die before they can use me to transmit them to a mammal host. By using complex regulatory mechanisms, they succeed in maintaining their numbers constant in order to keep me alive.

■ Under the influence

I have done my bit one more time ensuring the continuation of the next generation. My ovaries bear new traces of egg laying, adding to those that went before. Careful examination of the base of my ovarioles, which will be undertaken at the end of my reproductive life, will reveal the traces of 7 eggs. A lot of orange oocyte resorption matter also will be found, because there have been more and more yolk formation failures of late. My life as an adult tsetse fly is now one of a fly constrained by parasites. Thankfully, my larva offspring remains healthy.



A slight handicap in flying.

My flying capacity is reduced by 15% because part of the proline required by my wing muscles has been diverted by the trypanosomes to cover their own energy needs. In their epimastigote form, the trypanosomes swim up the salivary glands towards the hypopharynx to bind around the digestive tract. The transformation into metacyclic forms takes place in the salivary glands. Infectious metacyclic trypanosomes are now ready to be transmitted to my next host.

I now become exhausted while sucking the blood of my victims. I take less and less blood at each meal because the parasites are clogging my proboscis. I am forced to increase the frequency of my meals to satisfy my needs. This is a very effective propagation strategy on the part of the trypanosomes because I unwillingly inject them into a new mammal host with each bite. My spontaneous abortions occur with increasing frequency. The alternating rhythm of blood meals and healthy larvae production has been broken.

I even have the impression that I am losing some of my sensory capacities. Instead of choosing healthy animals, I tend more and more to feed on animals already infected with parasites, which puts me at risk of secondary infections from other trypanosome species with different itineraries and life cycles.



A cow infected with Nagana disease.

■ Taking a closer look

Trypanosomes belong to what is called the protist kingdom, which means that they are neither fungi, animals, nor plants. Modern phylogeny class them in the *Euglenobiontes* taxa, a group of protozoa in which trypanosomes rub shoulders with other parasites, Leishmania, along with green marine algae, euglenoids, and pseudocilia, which eat marine sludge!

The *Trypanosomatidae* family is divided into 8 genera of which only one, *Trypanosoma*, includes the trypanosome species pathogenic for humans and animals.

Each trypanosome species and sub-species is transmitted by specific tsetse fly species or sub-species which have special host targets.



Forms of trypanosomes.

Among the trypanosome species that transmit Nagana disease to animals, some are ubiquitous, others have marked preferences. In Africa, *Trypanosoma vivax* specifically infects ungulates such as pigs and cows, and certain wild animals such as elephants and the rare rhinoceros. *Trypanosoma congolense* affects cattle in particular, and *Trypanosoma brucei brucei* is very aggressive in domestic ruminants (cattle, goats, sheep), horses, donkeys, dromedaries, and domestic carnivores such as dogs and cats.

My preference for bovines leads me to think that I am carrying *Trypanosoma brucei brucei*, which causes Nagana disease in cattle, but I could be infected with other species or sub-species of trypanosomes depending on my food hosts. In that case, their cohabitation either translates into a kind of equilibrium or transforms itself into a relationship where one species dominates the other.

■ A herd in bad shape

I could easily have lived out my life without ever becoming infected by these parasites. Over 90% of my fellow flies never cross paths with a trypanosome and remain healthy during their entire life cycle. Unfortunately, I am part of the small percentage of vector tsetse flies, taken hostage by a parasite that knows how to exploit my energy metabolism to live and reproduce at my expense.

Most trypanosome species seem to use us, tsetse flies, as priority transmission agents because they know very well how to get around our natural immune system.

Some choose other vectors such as diptera, gadflies, stable flies, and even plant bugs, or other contamination paths. One species propagates itself through sexual transmission in horses, which then suffer from a venereal



trypanosomosis disease known as dourine.

A gadfly (left) compared to a stable fly (right).

In Latin America, one trypanosome species is even transmitted through the bite of vampire bats. Transmission can also take place accidentally via the placenta from a mother to her unborn child or through blood transfusions. Lastly, some trypanosomes exploit the carnivorous diet of some wild animals, such as the lions and hyena in Africa, to infect them through the intermediary of infected herbivores consumed by the

Trypanosomes are able to reproduce in the hundreds of thousands simply by dividing themselves into two every 7 hours. Manipulated by these pathogenic agents, I will unwillingly contaminate animals or people depending on the type of species inside me. In other words, these parasites do not hesitate to ruin the health of invertebrates and vertebrates to complete their own life cycle.



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Risky cohabitation:
the mammal and the trypanosome-infected fly.

Inside my body, trypanosomes are in what is called the procyclic phase of their development, while the mammals that I am going to infect will host the sanguinicole phase in their extracellular fluids.

To better adapt to their two successive hosts, these unicellular trypanosomes, equipped with a flagellum (a tail-like propeller used to move about) and with an undulating membrane, have a formidable capacity for morphological and physiological differentiation. I absorb them in their trypomastigote form. Only those which have a stumpy look are infective and will continue developing. They invade my intestines where they elongate, become uninfected, and rapidly multiply. The invasion advances. The trypanosomes transform themselves into another morphologically similar, uninfected form known as the epimastigote stage. Within one month, I am overtaken. The parasites reach my salivary glands where they again transform themselves into infective trypomastigotes ready to contaminate my future hosts.

With each blood meal, I introduce the infective, stumpy form of hundreds of trypanosomes into the body of my host. At the bite site, an inoculation chancre appears where they transform themselves into slender trypanosomes that are uninfected but can reproduce rapidly and invade the blood and lymphatic system of my victim. The victim reacts by mobilizing its immune system. The antibodies produced destroy nearly all of the trypanosomes. The survivors assume the short stumpy form and modify their surface antigens before changing again into a slender form which multiplies while waiting for the contaminated host to produce different antibodies. But again, some of the parasites survive and modify their antigenic proteins once more. This continues until the immunological resources of the host are exhausted. It seems that the *Trypanosoma brucei brucei* theoretically is able to vary its antigens in an unlimited manner to resist nearly all of the immune defences of a host. Each has 1,000 surface antigen coding genes which provide them with an equal number of protective disguises.



Different stages during the same life cycle.

When I observe the involuntary consequences of my fellow flies' blood meals on animals, the clinical picture is disastrous. Many cattle, some breeds of sheep, goats, and dromedaries, lose weight and hair, their eyes become watery, and they develop oedema. Their young suffer from a drop in their mother's milk production. Fewer offspring are produced because infected pregnant females tend to abort more frequently than usual.

■ Attacking a human

I often see in the distance men keeping watch over herds, women going to fetch water, and children bathing. One day, I even took a chance and bit an inattentive young boy who was walking under the cover of the forest near my roost, despite the high risk that I might develop a secondary infection from *Trypanosoma brucei gambiense* or even *Trypanosoma brucei rhodesiense* that infect people here in sub-Saharan Africa.



Once upon a time, there was a man.

Although the odours of humans are less attractive than those of cattle, I am so worn out by my increasingly exhausting quest for food and successive pregnancies which often end in abortion, that I opt for an easy target, a man taking a nap beside a river during the hot time of day. As his back is difficult to reach, I discretely install myself on his elbow. My bite, which is less painful than that of a horsefly, should not wake him.

In 30 seconds, the job is done. I leave satiated, relieved from having emptied my salivary glands, some parts of which are now showing necrosis.

If the man discovers a funnel shaped bite mark on his elbow in a few days, this will be a sign that I just transmitted to him several hundred *Trypanosoma brucei gambiense* in their infective, metacyclic form. He then will be infected with the chronic form of sleeping sickness. I feel less guilty considering that if he had lived further east or in southern Africa, he would probably have been the victim of the acute form, which is nearly always fatal for infected people, because my tsetse fly cousins belonging to the *morsitans* group would have infected him with *Trypanosoma brucei rhodesiense*.

As for me, I am feeling a little better, the nutritive value of this new meal having been particularly reinvigorating.



A sick person in the shade of a hut.

I imagine the future of this man who appears still to be young. In the first phase of the illness, which develops slowly, it will be easy to recognize the disease from the swellings on his face and the ripe, prune-shaped ganglia that will form at the nape of his neck and the base of his throat. While tired, he will still appear fit. But in fact, his future is darker than mine: he will suffer from irregular fevers, insomnia, poor reflexes, liver, kidney and cardiovascular disorders.



In the final phase of sleeping sickness.

When the trypanosomes invade his brain, the inflammation will cause stupor, apathy, mood swings, paralysis, trembling, uncoordinated movements and poor thermoregulation. But the sleeping sickness disease will really come into its own when it starts to affect his sleep-wake cycle: he will pass from a 24 hour cycle to a 90 minute one. He may even fall asleep while walking or eating. When this happens, his end will be near. In most cases, divided between intense pain and delirium, he will not be aware of what is happening to and around him.

■ Nothing to be proud of

I realize now that the village located near the forest gallery that I use as my resting place must be filled with victims of sleeping sickness.

Some of the inhabitants seem to be careless and lazy, their faces shorn of expression. They walk with an unsteady gait, as if they had been drinking alcohol or are very tired. Sometimes they become very agitated and waste massive amounts of energy in their delirium. Their immediate family and neighbours are worried, and ask themselves if the symptoms are contagious and, above all, if those who are sick are victims of a curse.

As I like to return to bite in the same place where I have fed before, guided by my circumstantial memory, I infect my victims' family, one after another. This is why some suspect it is contagious. The witch doctor, whose potions to diminish the facial swellings, and whose services are very expensive for the sick people's families, does not deny these superstitions in order to maintain his power. During this time, *Trypanosoma brucei gambiense* parasites begin to flourish inside my own insect body and inside the bodies of the infected people. They probably have achieved dominance over the *Trypanosoma brucei brucei*.

Under the village tree, the old people speak of a time in the distant past when doctors regularly visited to identify the sick and treat them with arsenic based drugs. Some of those suffering from sleeping sickness died from the remedy, most were cured, but could be infected again.

■ The beginning of the end

The infectious state in which I find myself has not become worse. The trypanosome population inside my body remains more or less stable but continues to divert a good part of my vital energy for its own needs. I notice that it is taking longer and longer to produce a fertilized egg. The suckling of the larva developing in my uterus tires me more than it used to. My resting periods during the day are longer and my meals, although more numerous, are less invigorating.

The edges of my wings are becoming frayed, which diminishes my flight lift and disturbs my manoeuvres. I sometimes even miss landing on my roost, which is extremely distressing. My sensory receptors are also becoming worn out: the mechanoreceptor bristles are breaking, the chemoreceptor wells are becoming clogged. I try to compensate with the experience I have gained over the course of my adult life to avoid becoming involved in dangerous situations. But how much longer can this go on?



Being old and sick does not stop me from seeking prey.

I now sometimes forget to clean my antennae, mouth pieces and feet. I therefore am less and less aware of changes in my nearby environment and I am less discriminating when choosing my prey. I indiscriminately bite healthy children, sick men, young calves and old bulls, and even an old sow. Little by little, I draw closer to habitats transformed by humans because it is easier to find victims in such areas compared to elsewhere. The more infected I become, the more I infect others with indifference.

My ageing body is but a tool serving the ambitions of a manipulative parasite whose survival strategy is the exact opposite of mine. I have become a flying hypodermic syringe serving the trypanosomes. While for me, the dozen or so offspring which I managed to bring into the world are a sign of success, the parasite considers that the few hundred trypanosomes transmitted to random mammals during my feeds were worth the loss of the thousands which died before being transmitted or which will die with me.



Closer and closer to the village where hosts are numerous.

■ An unexpected conclusion

As I approach my 100 day birthday, I no longer notice the morphological, metabolic, or biochemical changes of the trypanosomes that either are preparing to pass into a mammal's body or, in contrast, are just coming from one.



An involuntary link with humans.

I do not care if they practice oxidative phosphorylation respiration to survive in my oxygen deficient interior, or that they have adapted to my body temperature of between 20 to 25°C, that they have stretched into slender forms, or that they have lost their coat of glycoproteins made of 10 million molecules. I spend my nights tucked into a discrete spot under the eaves of a hut, letting the mosquitoes, those nocturnal vampires, rule the air. Everything is going well until a villager, irritated by being woken by these other blood suckers, reacts furiously to chase away the intruders.

In the general panic, I reflexively take off and leave the hut by an opening filled with moonlight and find a peaceful young calf whose legs strike me as an excellent place to roost until morning. Unfortunately, too old to notice from a distance the insecticide that humans have placed on this part of the calf's body, I land, unaware of the danger. When I sense an uncomfortable feeling, it is already too late. The insecticide has penetrated my cuticle.

In the seconds that follow, I lose muscle coordination and fall onto my back on the ground. My wings and feet jerk spasmodically. My parasite riddled body is particularly sensitive to this neurotoxin. A large cockroach runs towards me, detects the insecticide with a brush of its antenna, and turns away, suspicious.

Saved? My senses shut down, one after another. I have the impression that the parasites are panicking inside my condemned body. I can no longer see. The heart-shaped vessel which serves as my heart freezes. My ganglia refuse to transmit any more electric impulses. My muscles disconnect. I regurgitate some saliva and lose all sensation in one last spasm of silent pain.



The last moments after a fatal intoxication.

As the rising sun ends my last night in Africa, will the chickens wandering around the hut eat my inert body for breakfast? I will never know.

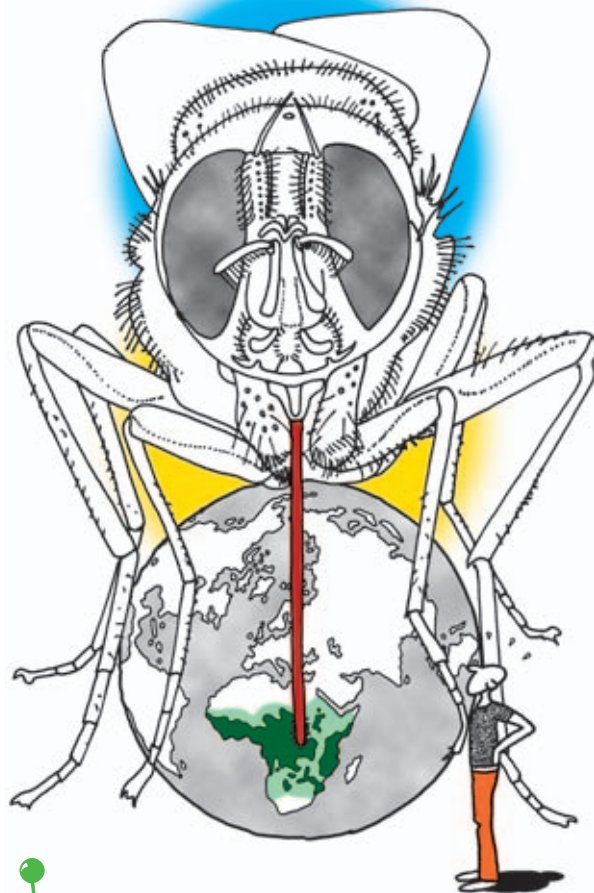


**A disease shared
between
animals and humans**



■ The high cost of cohabitation

Of the **42** poorest countries in the world,
32 are African and they host the
tsetse fly vector of trypanosomes
in **260** relatively permanent infections sites,
spread over an area of at least
7 million km², thus threatening
50 million cows,
230 million sheep,
40 million goats with animal
trypanosomosis, leading every
year to the death
of **3** million head of cattle, the loss of
500 000 tonnes of meat, and
1 million tonnes of milk.
The reduction in animal draft power
by **10** % in Africa, and as high
as 50 % in Asia
and up to **50** % drop in livestock productivity,
costing **5** billion euros a year for the African
economy.
Of the **60** million people who are exposed to
human trypanosomosis,
3 million infected carriers suffer with
500 000 more people infected yearly,
of whom **95** % are not diagnosed nor treated.
Consequently **90** % of them ultimately die
from this disease, and the remaining
10 % undergo medical treatment,
which can be so hard to withstand
that **5** % of them die as a result of this
therapy, adding to the
100 daily reported deaths due
to sleeping sickness.



All of this because, across almost two-thirds of the immense African continent, a small fly, which is not any bigger than a housefly, is exploited by an erythrocyte-sized parasite which subsequently reproduces in the bodies of humans or domestic animals, often leading to their deaths after very painful suffering.

■ SOS Initiative, Uganda

The Stamp Out Sleeping sickness (SOS) initiative is a public private partnership launched in Kampala, Uganda in October 2006. This partnership was formed in response to an emergency situation arising in a number of districts of Northern Uganda where the two strains of Human African Trypanosomiasis (HAT) -- also known as "sleeping sickness"-- threaten to converge.

The partnership consists of: The Ugandan Government represented by COCTU (Co-ordinating Office for the Control of Tsetse and Trypanosomiasis in Uganda), Makerere University, Edinburgh University, IKARE and Ceva Santé Animale.

The partnership succeeded in its original objective of mass-treating cattle in the area in order to prevent the terrible health consequences of a "convergence". Since 2009 Ceva and IKARE have together with local partner High Heights taken the initiative a step further by focussing on creating sustainability through the roll-out of private veterinary services and products to these communities affected by sleeping sickness.

Over 400,000 cattle have been treated to date in the target area. 11 graduate veterinarians, their assistants and a further 150 field workers or spray persons (who carry out routine spraying of cattle) have established their own private businesses to provide both tsetse and tick control and other animal health services to these communities.

The veterinarians became known as the "3V vets" because of the Ceva products they use and promote in their shops to protect cattle from infection - Vectocid, Veriben and Veridium.

The SOS initiative has achieved strong branding locally as well as at an international health community level with positive recognition of the benefits brought by the initiative.



For further information visit:
<http://www.stampoutsleepingsickness.com/>

■ IKARE Ltd

IKARE Ltd (pronounced "I care") is a UK registered charity applying venture philanthropy principles to the causes it supports. In so doing IKARE aims to overlay private equity investment techniques and general business expertise and practices into its causes or "investees", thereby contributing more than funding, and seeking measurable and sustainable societal impact.

The principles of venture philanthropy can be summarized as follow:

- High engagement; support of few social enterprises/ investees at a time;
- Organizational strengthening at investees;
- Tailored funding/financing for each social enterprise supported;
- Non-financial support as needed;
- Involvement of networks;
- Multi-year support provided;
- Performance (societal impact) measurement.

Starting small scale and focused in 2006, IKARE's resources have been fully dedicated to the SOS initiative, launched in Kampala, Uganda, in October of the same year. Having initially funded mass-treatments of cattle in Northern Uganda in an effort to stop the spreading and convergence of two different strains of sleeping sickness IKARE is currently engaged in rolling out private veterinary practices into these rural and previously under-served areas of Uganda.

This roll-out is co-ordinated and managed through the IKARE supported local partner High Heights Services Limited. To date, 11 private veterinary practices have been initiated, in turn creating jobs also for more than 150 so called spray persons which offer in-field spraying services on a commercial basis to farmers.

Regular training is provided, with the financial and technical support of IKARE and Ceva, both to the veterinarians as well as the spray persons to strengthen them in their roles and improve the quality of their services. IKARE's main donor is IK Investment Partners providing both financial as well as non-financial support.



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**IK Aid and Relief Enterprise Ltd
(IKARE Ltd)**

Brettenham House
5, Lancaster Place
London WC2E 7EN
England

Tel: + 44 20 7304 4300 - Fax: + 44 20 7304 4320
Main contact: Anne HOLM RANNALEET
Mobile: + 46 70 378 9550
Email: anne.rannaleet@ikininvest.com

■ Ceva Santé Animale

Together, beyond animal health

"The concept of having Ceva at the centre of One World, One Health is key. I think its key for people to understand that we don't work only for money or to sell some vet products but we are working to contribute to the fact that people will live better in the future, all over the world". Marc PRIKAZSKY, Chairman and CEO, Ceva Santé Animale.

The role of the animal healthcare industry has a significance which extends far beyond the traditional limits of the veterinary field. Early pioneers of veterinary medicine, such as, Edward JENNER and Louis PASTEUR worked in both animal and human medicine realizing the importance of "One Medicine". Once again, the importance seeing health both on a global and holistic basis has become evident. Ceva's mission summarized in our slogan "**Together, beyond animal health**" recognizes this and has three fundamental principles, all of which address concerns over broader social issues:

- We are dedicated to **combating zoonoses**, diseases which are transmitted between animals and humans and which carry the threat of serious pandemics, particularly in a world of ever-increasing mobility. Our founding and long term membership of the "Stamp Out Sleeping Sickness programme" in Uganda is one example of our commitment. The production of this booklet together with CIRAD and IKARE is another example of how together with both the scientific and local communities we can improve our knowledge and therefore help fight such socially catastrophic diseases as trypanosomiasis.

- The issue of **ensuring food resources and security** is also vital. At the current time not everybody in the world has access to sufficient quantities of animal protein. Improving the health of cattle in Africa through better control of tsetse and trypanosomiasis, means that not only is more meat produced but draft oxen plough more land and more crops are produced.

- The **essential link between humans and animals** has always been evident in rural communities and is now playing a new role in our increasingly urbanized society. Companion animals contribute significantly to our own emotional and physical health and in return we have a duty to protect their own well being.

This threefold mission is an attainable ideal, but it nonetheless demands enormous means and motivation, beyond the limits of what one company can achieve alone.



Ceva Santé Animale

10, avenue de la Ballastière. BP. 126
33501 Libourne - France

Tel: 33 (0)5 57 55 40 40
E-mail: contact@ceva.com
Web: www.ceva.com

■ CIRAD's contribution

CIRAD (*Centre de coopération internationale en recherche agronomique pour le développement*) is a French research institution specialized in agricultural research in developing countries and French territories overseas. CIRAD favours carrying out research in partnerships to respond to the challenges facing agriculture and international development, and works in over 90 countries.

Sustainable development is the driving force behind CIRAD's activities around the world. This approach takes into account the long-term ecological, economic, and social consequences of transformation processes of societies and territories in developing countries. CIRAD intervenes through research, experiments, training, communication and innovation activities, and the provision of expertise. Its skills in life, social, and engineering sciences are applied to food and agriculture, natural resource management, and social issues.

As part of its quest to disseminate scientific culture, CIRAD gave its SAVOIRS team (Service d'appui à la valorisation opérationnelle de l'information sur la recherche scientifique) the task of developing and testing new communication vectors and original narrative forms to transfer and share with diverse audiences scientific and practical knowledge on a variety of topics.

The projects are carried out in partnerships. Among the forty institutions with which the team works, Ceva Santé Animale is distinguished by its contribution to three compilivres®, "**Le dromadaire pédagogique**" (2002), "**La mouche tsé-tsé pédagogique**/The educational tsetse fly" (2005) and "**La planète des bactéries**" (2007), two educational handbooks, "**La grippe aviaire, l'influenza aviaire**" (2006) and "**Bird flu avian influenza**" (2006) and a scientific story, "**Journal intime d'une mouche tsé-tsé**" (2008).

In 2012, Ceva Santé Animale and IKARE provided support for the English version of "**Journal intime d'une mouche tsé-tsé**", which has been entitled, "**100 days in the life of a tsetse fly**". This contribution was part of their mission to raise awareness in Uganda, among isolated African populations such as livestock farmers as well as donors, about the fight against tsetse flies infected by pathogenic trypanosomes which cause Nagana disease in cattle and sleeping sickness in humans.



CIRAD-SAVOIRS
DIRECTION RÉGIONALE LANGUEDOC-ROUSSILLON
TA 178/05 - Avenue Agropolis
34398 Montpellier Cedex 5 - France

Tel: 33 (0)4 67 61 57 88 / Fax: 33 (0)4 67 61 59 73
E-mail: espace.idees@cirad.fr
Web: <http://savoirspartages.cirad.fr>

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